Newer Potential Biomarkers in Prostate Cancer

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Prostate-specific antigen (PSA) screening has led to a significant rise in the number of men diagnosed with prostate cancer and an associated increase in biopsies performed. Despite its limitations, including a positive predictive value of only 25%-40%, PSA remains the only generally accepted biomarker for prostate cancer. There is a need for better tools to not only identify men with prostate cancer, but also to recognize those with potentially lethal disease who will benefit from intervention. A great deal of work has been done worldwide to improve our knowledge of the genetics behind prostate cancer and the specificity of PSA by developing assays for different PSA isoforms. Common genetic alterations in prostate cancer patients have been identified, including CpG hypermethylation of GSPT1 and TMPRSS2:ERG gene fusion. Serum and urine detection of RNA biomarkers (eg, PCA3) and prostate cancer tissue protein antibodies (eg, EPCA) are being evaluated for detection and prognostic tools. This article reviews some of the promising developments in biomarkers.

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Prostate cancer is the most common cancer diagnosed in men today. The lifetime risk of being diagnosed with prostate cancer is approximately 16%, and more than 218,000 men in the United States will be diagnosed this year alone.[1] Since the introduction of prostate-specific antigen (PSA) testing in the late 1980s, prostate cancer diagnoses have increased, even as mortality rates for prostate cancer have declined. Over the past few years, there has been increasing recognition that not all men diagnosed with prostate cancer require treatment. Indeed, the 5-year survival for prostate cancer is over 98%. The landscape for

management of the disease has further changed with the recognition that many men diagnosed with low-risk prostate cancer (organ-confined, Gleason 6 prostate cancer) will not require definitive therapy for their cancer due to the low risk of morbidity and mortality. Accordingly, so-called active surveillance protocols are being implemented and studied in many centers for men who fit this low-risk profile.

At present, the only widely accepted screening tools for prostate cancer are prostate-specific antigen (PSA) and digital rectal examination. PSA is prostate specific, but not prostate-cancer specific. The positive predictive value of a PSA > 4.0 ng/mL is only 25% from a pooled meta-analysis of PSA studies.[2] Many urologists now use a PSA cutoff of 2.5 ng/mL, which increases the detection of prostate-cancer cases but also leads to a significant number of additional biopsies performed. Some investigators question the role of PSA as a screening tool altogether, arguing that it leads to a large number of unnecessary prostate biopsies and probably a large number of potentially unnecessary therapies without significantly impacting on cancer-relative survival. Randomized trials are underway in both Europe and the United States to evaluate the efficacy of PSA screening.

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PSA screening will continue to be used in clinical practice while we await the results of these studies, but in light of these controversies all would agree that we need to develop better tools for identifying not just men with prostate cancer, but men with potentially lethal prostate cancer who will benefit from intervention. As our knowledge of the genetics behind prostate cancer grows and our ability to perform rapid translational research increases, we have witnessed the development of multiple new tests that may enhance our diagnostic accuracy. In this article, we will review some of these biomarkers.

PSA Isoforms

A number of different PSA isoforms have been identified, including free PSA, proPSA, and BPSA. These PSA isoforms, or related PSA proteins, have been evaluated for the ability to predict prostate cancer. Extensive discussions of the biology of these isoforms are well summarized in other places beyond the scope of this review. Briefly, free PSA (fPSA) has established its role clinically with its ability to increase the specificity of PSA testing,[3] especially in men following a negative prostate needle biopsy. The isoforms BPSA and iPSA, although showing promise in some series, have failed to demonstrate consistently improved specificity over PSA and fPSA testing for the detection of prostate cancer. The other recently elucidated isoform, proPSA, has shown some promise.

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ProPSA is the precursor protein for PSA and contains a 7 amino-acid propeptide that is truncated in various ways. Elevated levels of the various truncated forms of the proPSA protein have been associated with prostate cancer.[4] A study of men undergoing prostate biopsy with PSA levels between 4.0 and 10.0 ng/mL, evaluated 3 isoforms of proPSA along with PSA and fPSA for their ability to predict prostate cancer.[5] Higher total proPSA levels were associated with prostate cancer but did not improve upon the specificity of total or fPSA alone. When considered together, however, a model including proPSA, PSA, and fPSA was superior to any of the individual tests. At a sensitivity of 95%, the combined model had greater specificity (37%) than PSA (15%) or fPSA (27%) alone.

In a large, multicenter trial of 2005 men (1046 with prostate cancer), the (-5, -7) proPSA isoform was evaluated against fPSA and total PSA.[6] In men with low PSAs (2.0-4.0 ng/mL), the area under the receiver operating characteristic curve (ROC) was not significantly better for this proPSA isoform or the ratio of proPSA:PSA compared with total or the free PSA:total PSA ratio. In the PSA range of 4.0-10.0 ng/mL, the proPSA:fPSA ratio had a better area under the ROC compared with total PSA (0.67 [95% CI, 0.65-0.68] vs 0.53 [95% CI, 0.52-0.55]), but added no diagnostic information over the fPSA:total PSA ratio (0.69; 95% CI, 0.67-0.70). Recently, proPSA was tested for its ability to distinguish between aggressive and non-aggressive prostate cancer. In a study of 376 men with prostate cancer undergoing prostatectomy, the ratio of (-5, -7) proPSA:fPSA was associated with higher Gleason grade ($P = .001$) and non–organ-confined disease ($P < .0001$).[7] Further investigation

into the ability of proPSA to detect not only prostate cancer but also more aggressive prostate cancer is underway.

Human Kallikreins

A number of kallikrein genes have been identified on chromosome 19, of which PSA is a member (hK3). A related serine protease and kallikrein is hK2. The gene was cloned, the protein isolated, and a serum assay developed. Some initial studies looking at serum levels of hK2 suggested it to be a potential marker for higher Gleason grade or stage,[8] but subsequent work showed only a marginal improvement in the value of serum hK2 levels when traditional variables are considered.[9]

The remainder of this review will focus on genomic-based biomarkers and novel prostate-tissue antigens.

DNA Biomarkers

Epigenetic Markers

Hypermethylation of cytosine guanine (CpG) dinucleotide islands at gene promoter regions of tumor suppressor genes has been recognized for a number of tumors as an important event in tumorogenesis, including prostate cancer. CpG hypermethylation is considered to be an initial step in prostate cancer development. A number of different candidate genes have been evaluated, and the most consistently hypermethylated in prostate cancer patients is the glutathione S-transferase pi (GSTP1) gene. This gene was analyzed initially in tissue as a marker to distinguish benign from malignant tissue. Later, GSTP1 hypermethylation was studied in urine sediment as a non-interventional test for determining the need for prostate biopsies.[10-12]

Other candidate genes have been examined for hypermethylation along with GSTP1. Two recent studies looked at a panel of 10 candidate genes (APC, DAPK, ECDH1, GSTP1, MGMT, p14 [ARF], p16, RARβ2, RASSF1a, and TIMP3).[13,14] The first study compared urine sediment from 52 prostate cancer patients undergoing radical prostatectomy with that of 91 age-matched controls.[13] All 52 prostate cancer patients had at least 1 hypermethylated gene, and 80% had 3 or more hypermethylated genes. The 4 most common genes involved were GSTP1, p16, ARF, and MGMT. All 52 prostate cancer patients had at least 1 of these genes hypermethylated, and none of the 91 controls had hypermethylation of any of these genes. In the second, more recent study, 95 patients undergoing radical prostatectomy and 38 age-matched controls who had negative prostate biopsies submitted urine after prostatic massage.[14] Eight of the loci had increased methylation in cancer patients over the controls ($P < .05$). The 4 genes with the greatest difference (GSTP1, APC, RASSF1a, and RARβ2) had sensitivity for prostate cancer detection of 86% and a diagnostic accuracy of 89%.

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Gene Fusion Proteins

Gene rearrangements are associated with a number of cancers, especially leukemias and lymphomas, and recent studies have uncovered gene rearrangements in patients with prostate cancer as well. One such rearrangement involves the ETS-related genes (ERG) at 21q22.2 and ETV1 at 7p21.2 with TMPRSS2 (21q22.3).[15] ERG and ETV1 are both ETS transcription factor genes, and TMPRSS2 is an androgen regulated gene. The fusion of these genes is seen in 40%-80% of prostate cancer patients, approximately 20% of prostatic intraepithelial neoplasia (PIN) cases, and rarely in benign prostatic tissue.[16]

In a study of 252 men with stage T1a/b prostate cancer followed for a median of 9 years, TMPRSS2:ERG gene fusion was more commonly associated with Gleason scores > 7 (41% vs 12%; $P = .01$) and more prostate cancer deaths and/or metastatic disease development (53% vs 23%; $P = .03$).[17] In univariate analysis, the cumulative incidence ratio was 2.7 (95% CI, 1.3-5.8; $P < .01$) for the association between the TMPRSS2:ERG gene fusion and prostate cancer specific death and/or metastatic disease. After controlling for Gleason score, however, this cumulative incidence ratio did not reach statistical significance (CIR = 1.8; 95% CI, 0.6-5.3; $P = .2$).

A urinary test for the detection of TMPRSS2:ERG fusion products has been developed using RNA amplification and quantitative PCR.[16] In the pilot study of 19 patients with prostate cancer, urine was collected after prostatic massage. Forty-two percent of the patients had the TMPRSS2:ERG gene fusion detected, consistent with what is found in the tissue analysis data. The researchers confirmed with fluorescence in situ hybridization (FISH) analysis on the radical prostatectomy specimens the

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presence of the TMPRSS2:ERG gene fusion in a subset of the patients. Further work is required, and the assay used was only directed toward 1 of the TMPRSS2:ERG gene fusion isoforms, although this isoform is the most commonly detected (80%-95%) in patients with TMPRSS2:ERG gene fusion. Perhaps these fusion genes ultimately will serve more as targets for therapy than as biomarkers, much as was the case with myelogenous leukemia.

Loss of Heterozygosity

Loss of heterozygosity is a frequent genetic abnormality in prostate cancer that has been described at multiple chromosomal sites. A group recently worked to develop a urinary marker of loss of heterozygosity looking at 14 microsatellite markers at 7q31, 8p22, 12p13, 13q14, 16q23.2, and 18q21.[18] In 99 patients undergoing prostate biopsy (58% found to have prostate cancer) the genomic DNA was obtained following prostatic massage. Compared with a free:total PSA cutoff < 15% from the same cohort, detecting loss of heterozygosity had superior sensitivity (87% vs 44%; $P = .002$) but worse specificity (55% vs 76%; $P = .006$). In patients who underwent radical prostatectomy, the loss of heterozygosity was confirmed from the prostatic tissue with a concordance of 86%. This approach still requires much investigation to determine its biomarker potential.

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RNA Biomarkers

PCA3

PCA3 is a non-coding RNA previously known as DD3 that is very prostate specific.[19] PCA3 is highly expressed in both prostate-cancer specimens and prostate-cancer metastasis with a more than 60-fold upregulation over benign prostate tissue.[20] PCA3 mRNA levels can be measured in the urinary sediment after prostatic massage. Three diagnostic tests have been developed that measure PCA3. The first was a dual time resolved fluorescence-based RTPCR assay used in the primary research in the Netherlands,[20] and the second was uPM3™ (Bostwick Laboratories, Glen Allen, VA), a lab-developed test using nucleic acid sequence based amplification.[21] The third is APTIMA® (Gen-Probe Incorporated; San Diego, CA), which uses transcription mediated amplification[22] and is the only reagent currently available commercially.

A tabulation of various PCA3 urine studies is given in Table 1. In each case, the voided urine after prostatic massage was collected, and the PCA3 level was measured and normalized to the urine PSA level to create a PCA3 score. Appropriate PCA3 scores provided a relatively high level of sensitivity (range 61%-82%) and specificity (76%-89%) with an area under the ROC of 0.70-0.87.[23] PCA3 scores from prostatic secretions, rather than urine after prostatic massage alone, have also been evaluated.[23] In an analysis of 67 men undergoing prostate needle biopsy, PCA3 scores were compared between those with and without prostate cancer detected (34% detection rate). The PCA3 scores were more significant in the prostatic massage specimens (73 vs 18; $P < .001$) as compared with the voided PCA3 scores (48 vs 19; $P = .006$), but these differences were not statistically different ($P = .19$). Given the variability in obtained adequate volumes of expressed prostatic secretions, the post-prostatic massage urine assays likely hold more promise.

More recently, a multi-institutional study of 534 patients (33% with prostate cancer) undergoing prostate biopsy compared PCA3 scores with PSA for predicting prostate cancer.[24] Using a PCA3 score of 58 as the cutoff, PCA3 had an area under the ROC of 0.66 (95% CI, 0.61-0.71) compared with 0.57 (95% CI, 0.52-0.63) for PSA. Higher PCA3 scores also correlated with increasing risk of cancer detection. PCA3 has also been evaluated recently for men undergoing a second prostate biopsy after a previous negative biopsy.[25] In 233 men undergoing repeat biopsy (of whom 23% were found to have prostate cancer), PCA3 scores had an area under the ROC of 0.68 (95% CI, 0.60-0.76) with a

Table 1
Studies on PCA3

Authors	Year	PCA3 Test	Number of Patients	Area Under ROC
Marks et al[25]	2007	APTIMA	226	0.68 (0.60-0.76)
Groskopf et al[22]	2006	APTIMA	143	0.75 (0.57-0.92)
Fradet et al[21]	2004	uPM3	443	0.86 (0.82-0.89)
Tinzl et al[26]	2004	uPM3	201	0.87 (0.81-0.92)
Hessels et al[20]	2003	Fluorescence-based RT-PCR	108	0.72 (0.58-0.85)
van Gils et al[24]	2007	Fluorescence-based	534	0.66 (0.61-0.71)

ROC, receiver operating curve.

maximal sensitivity and specificity of 58% and 72%, respectively. Although showing great promise, PCA3 still requires additional work. The literature on PCA3 uses a number of different assays and thresholds for prostate-cancer detection, raising questions of reproducibility and standardization.

Alpha-methylacyl CoA Racemase

Alpha-methylacyl CoA racemase (AMACR) is located at 5p13.3, a gene region found to be important in prostate cancer in several genome-wide scans.[27] AMACR gene polymorphisms are found to cosegregate in prostate cancer families (logarithm of the odds 3.78; $P < .001$).[28] AMACR functions in the oxidative metabolism and biosynthesis of branched chain fatty acids found in dietary red meats and dairy products.[29] Both red meat and dairy products have been associated with increased prostate cancer risk.[30] The interaction between the genetic aspects of AMACR and the modifiable environmental factors of dietary intake of fats offers an exciting possible mechanistic explanation for a multistep development of prostate cancer. A meta-analysis of expression microarrays (Figure 1) found that AMACR is consistently overexpressed in prostate cancer with high specificity (79%-100%) and sensitivity (82%-100%).[31] In fact, antibodies against AMACR are commonly used for immunohistochemistry analysis of prostate biopsies to help distinguish benign from malignant tissue. Elevated levels of AMACR mRNA have been detected in prostate cancer patients by RT-PCR from serum, urine, and prostatic secretions although the work has been limited to small series and primarily limited to proof of principle.[36,37] Circulating levels of AMACR protein are quite low, making development of a serum test difficult, but some investigators have demonstrated AMACR protein levels in urine by

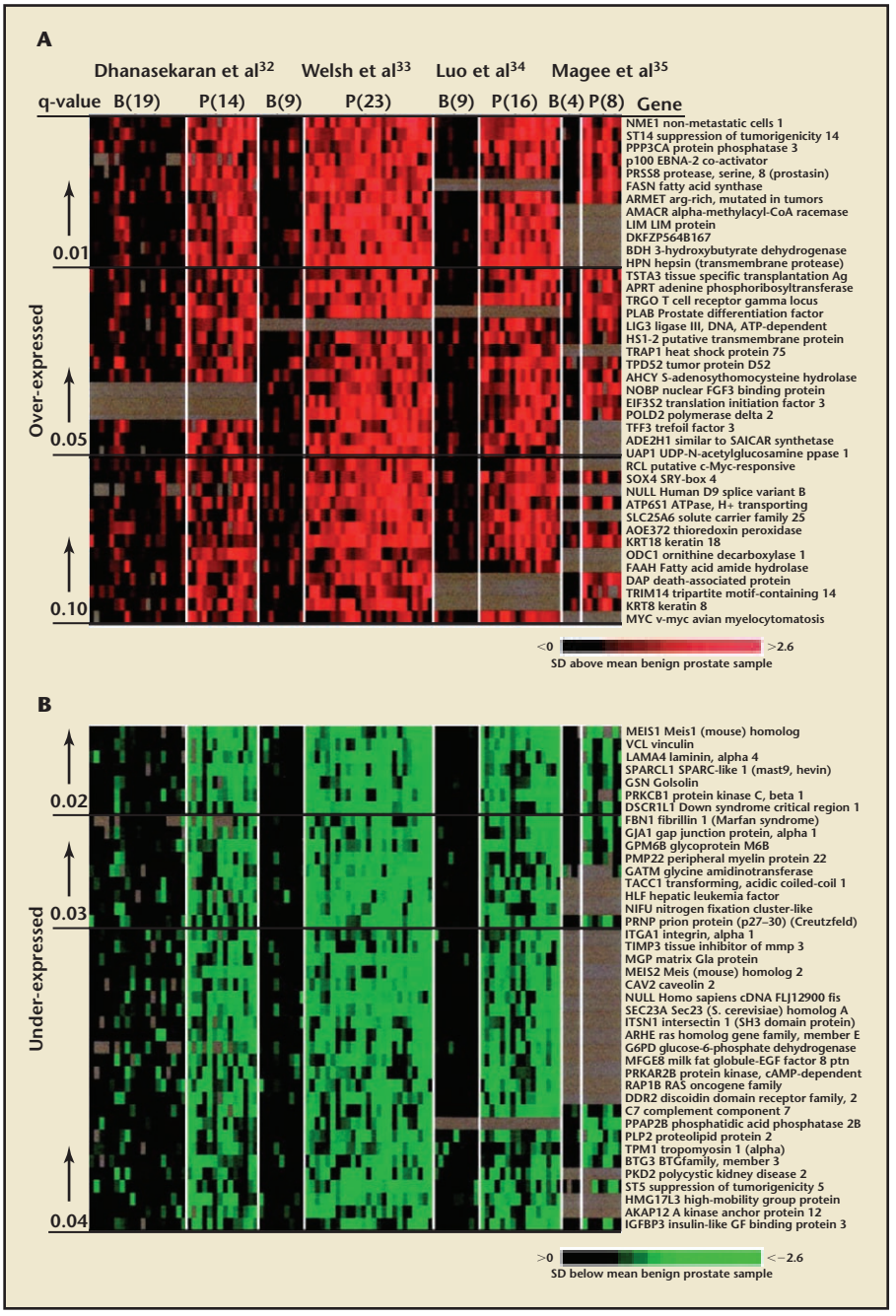

Figure 1. *A cohort of cross-validated genes identified by meta-analysis of prostate cancer gene expression profiles. Eisen matrix representation of genes consistently differentially expressed between clinically localized prostate cancer (P) and benign prostate tissue (B) across 4 independent microarray studies.*[32-35] Each column *represents an individual sample (number of samples is in parentheses), and* each row *represents a specific gene. Within each study, the data were normalized so that the mean expression level of the genes in the benign prostate specimens equaled zero and the SD = 1.* **(A)** *40 genes with the lowest q value for overexpression.* Red *intensity level indicates degree of overexpression, whereas* black *indicates equal or lower expression than the mean benign sample (see scale).* **(B)** *40 genes with the lowest q value for underexpression.* Green *intensity level indicates degree of underexpression, whereas* black *indicates equal or higher expression than the mean benign sample (see scale).* Gray *signifies technically inadequate or not present in a particular study. q value thresholds are provided to the left of the matrix. Reprinted from Rhodes et al*[31] *with permission from the American Association for Cancer Research.*

Western blot analysis.[38] Additionally, 1 group was able to detect higher levels of antibodies to AMACR in patients with prostate cancer compared with those without cancer with a sensitivity and specificity of 62% and 72%, respectively.[39] Further studies of AMACR are underway to determine its clinical applicability.

Antigen Biomarkers

Prostate Antigens

Several prostate-specific antigens in addition to PSA have been evaluated for detection of prostate cancer, including prostate-specific membrane antigen (PSMA), prostate stem cell antigen (PSCA), and early prostate cancer antigen (EPCA) among others. Unfortunately, the results have been mixed for PSMA, and the data for PSCA and EPCA are limited. EPCA has shown promise as a nuclear structural protein associated with prostate cancer. Initially, EPCA antibodies were shown to have increased staining in prostate cancer patients' tissue over controls.[40] A serum immunoassay for ECPA antibodies has since been developed. In a small study of 12 cancer patients, using a cutoff of 1.7 absorbance at 450 nm, EPCA identified 92% (11/12) patients with cancer.[41] None of 16 healthy donors had EPCA levels above the cutoff, but 2 of 6 bladder cancer controls did have EPCA levels above 1.7 absorbance for an overall specificity of 94%. A second EPCA protein (EPCA-2) was recently identified. In a study with a diverse population of patients, 100 of whom had prostate cancer, and using a cutoff of 30 ng/mL at a concentration of 0.8 at the absorbance level, most of the men with prostate cancer were identified (sensitivity of 94%).[42] EPCA-2 outperformed PSA when analyzed by the area under the ROC (0.96 vs 0.77; $P < .001$), although this study was not in a screening population. The data published on EPCA continue to be very promising, but progress toward clinical implementation seems very slow so far.

The data published on early prostate cancer antigen continue to be very promising, but progress toward clinical implementation seems very slow so far.

Autoantibody Arrays

Similar to the detection of antibodies against AMACR in patients with prostate cancer, a broader panel of autoantibodies against prostate cancer tissue peptides has been explored. Using a library created from mRNA taken from 6 patients with clinically localized prostate cancer, researchers developed a 22-phage-peptide detector as determined from multiple procedures on protein microarrays.[43] In their analysis, the phage-peptide detector had a high specificity (88%; 95% CI, 78%-95%) and sensitivity (82%; 95% CI, 70%-90%). In addition, the area under the ROC for the detector was 0.93 (95% CI, 0.88-0.97), which was statistically superior ($P < .001$) to the ROC for PSA (0.80; 95% CI, 0.71-0.88). The investigators also applied the detector to patients with lung cancer, and 9 of 30 patients were misclassified as having prostate cancer, suggesting that there is cross-reactivity between different cancer autoantibodies. One puzzling problem in these data so far is that one cannot be sure that the needle biopsies themselves elicited these autoantibodies, because prostate tissue is well known to produce autoimmune reactions.

Conclusion

The PSA era has ushered in a significant rise in the number of men diagnosed with prostate cancer and resulted in a tremendous number of biopsies performed to rule out the disease. With detection rates of 25%-40%, and more than 200,000 incident cases of prostate cancer annually, the number of biopsies performed annually approaches 1 million. PSA testing has survived as one of the few biomarkers accepted for cancer detection, despite its obvious limitations and poor

Main Points

- Prostate-specific antigen (PSA) testing continues to have an important role in prostate cancer detection but it is limited by its poor specificity.
- PSA isoforms have improved our understanding of the biochemistry behind PSA and prostate cancer but have failed to demonstrate dramatic improvement in the detection accuracies.
- Common genetic alterations in prostate cancer patients have been identified, including CpG hypermethylation of GSPT1 and TMPRSS2:ERG gene fusion.
- Serum and urine detection of RNA biomarkers and prostate cancer tissue protein antibodies are being evaluated for detection and prognostic tools.

specificity. A great deal of work has been done across the world in an attempt to improve upon the specificity of PSA by developing assays for different PSA isoforms. This effort has improved our understanding of the biochemistry behind PSA and prostate cancer but has produced no dramatic improvement in the accuracy of detection. Nonetheless, a host of newer biomarkers, especially DNA and RNA biomarkers, are showing promise, and 1 or more of these may yet evolve into useful diagnostic and/or prognostic tools. Finally, more recent molecular inquiries suggest that even if the markers just discussed do not prove sufficiently useful, other more promising ones will surely come forth. ■

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